



## SciForum MOL2NET

### Identification of triterpenic saponin by HPLC-DAD-MS/MS in *Zornia brasiliensis*

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Received: / Accepted: / Published:

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**Abstract:** *Zornia brasiliensis* (Leguminosae) is a species popularly known as "urinária", "urinana" and "carrapicho" and its popular use is reported as a diuretic and for treatment of venereal diseases. It occurs in several states of Brazil, being abundant in the Northeast. Therefore, this study aims to contribute to the chemical knowledge of this species through an HPLC-DAD-MS/MS study for the rapid determination of saponins. Aerial parts of *Z. brasiliensis* were collected in Serra Branca, state of Paraíba. An exsiccata was deposited in the Herbarium Arruda Câmara (ACAM) of Campus I of UEPB. After drying and ground, the plant material was subjected to a 95% ethanol maceration for 72 hours, and this process was repeated four times to obtain the crude ethanolic extract (CEE). An aliquot of the CEE (100.0g) was subjected to vacuum liquid chromatography (VLC) with silica deactivated with hexane, dichloromethane, ethyl acetate and methanol solvents. An aliquot of the 50% acetate-methanol fraction was subjected to a specific methodology for the concentration of saponins. This fraction was then analyzed by HPLC-DAD-MS<sup>2</sup>, allowing the identification of a triterpenic saponin, suggesting to be the Soyasaponin IV, described for the first time for the genus *Zornia*. In this way, this work contributed to the chemistry *Z. brasiliensis* and corroborated with the chemitaxonomy of the family Leguminosae.

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**Keywords:** Soyasaponin IV; *Zornia brasiliensis*; Saponin.

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## 1. Introduction

*Zornia* (Leguminosae) has about 80 species in the world, with 41 representatives in America, 16 in Africa, 13 in Oceania and 7 in Asia [1-2], is the second largest representative of the *Adesmia* clade.

*Zornia brasiliensis* is a species popularly known as "urinária", "urinana" and "carrapicho"

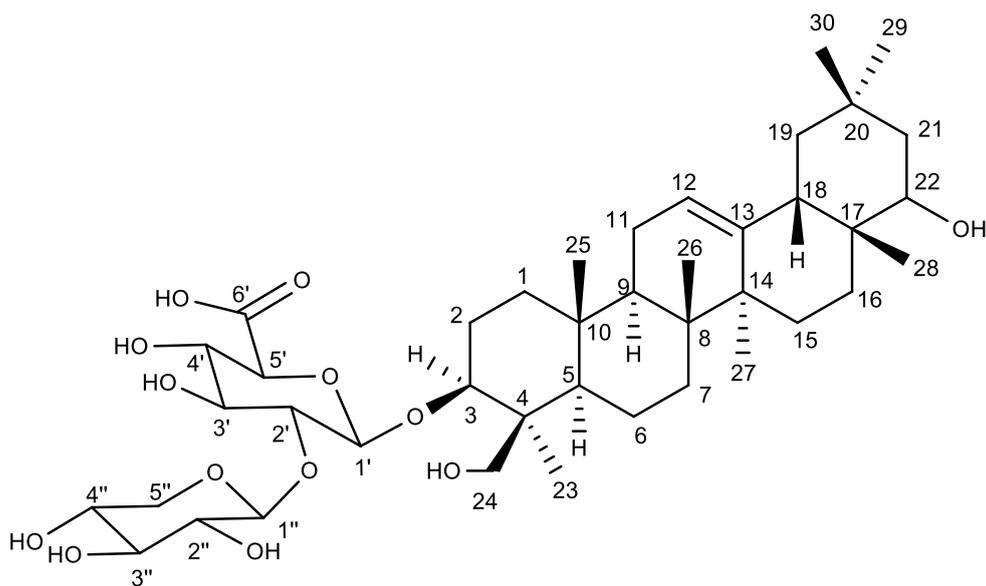
and its popular use is reported as a diuretic and for treatment of venereal diseases [3]. It occurs in several states of Brazil, being abundant in the Northeast. In view of this, this study aims to contribute to the chemical knowledge of this species through a study by HPLC-DAD-MS/MS for the rapid determination of saponins.

## 2. Results and Discussion

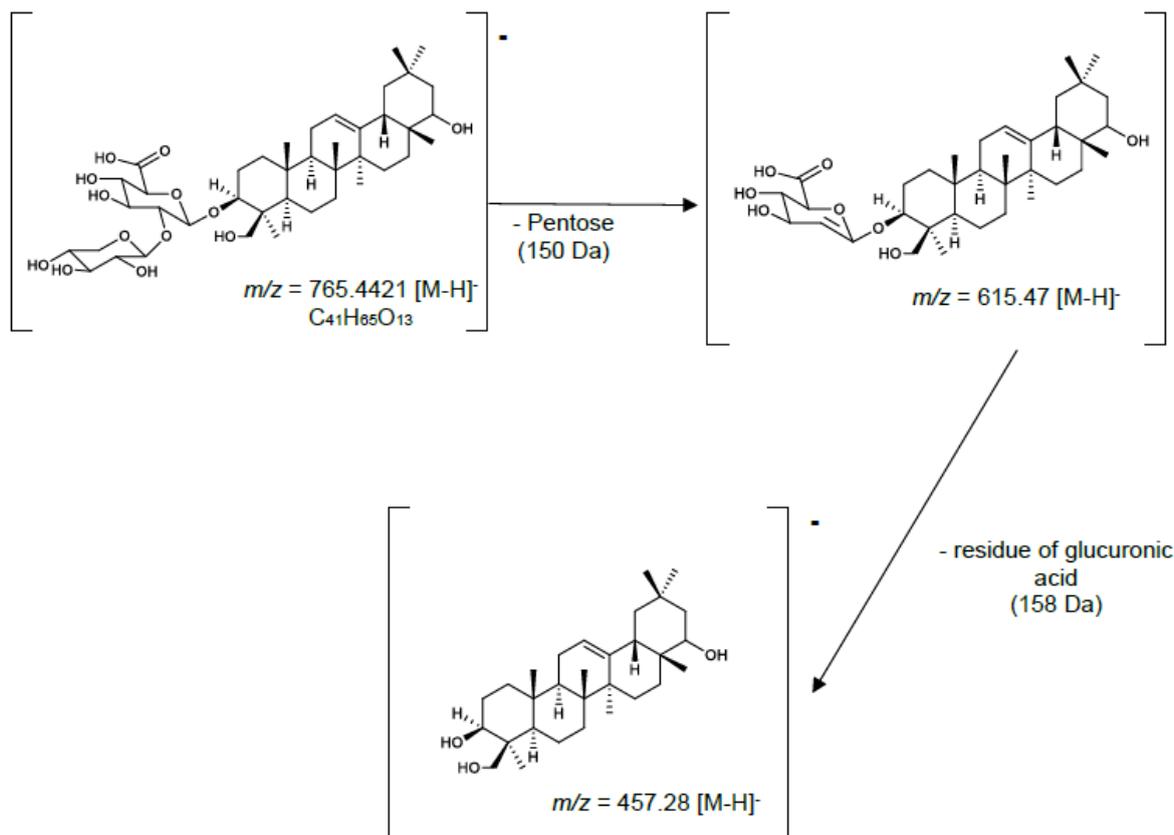
The FrST fraction was analyzed by HPLC-DAD-MS / MS. First, the low resolution HPLC-DAD-MS analysis was performed in MS and MS<sup>2</sup> mode so that the retention times, the peaks of the molecular ions and their respective fragments can be obtained. Then, high resolution HPLC-DAD-MS analysis was performed in MS mode, similarly, retention times, molecular ion peaks, can be obtained. In addition to the molecular formulas.

compatible with the molecular formula C<sub>41</sub>H<sub>65</sub>O<sub>13</sub>. A sequenced loss of sugars with fragments in *m/z* 615.47 and 457.28, compatible with the loss of a pentose-like structure (150 Da) and a residue of glucuronic acid (158 Da), respectively. The fragment with *m/z* = 457.28 was compatible with Soyasapogenol B, a triterpenic sapogenin, on the basis of these data it is suggestive that compound 1 is Soyasaponin IV [4].

Compound 1 showed a retention time of 32.5, *m/z* 765.4425 [M-H]<sup>-</sup> (*m/z* 765.4421, calcd)



**Figure 1.** Chemical structure of Soyasaponin IV.



**Figure 2.** Proposed fragmentation of Soyasaponin IV.

### 3. Materials and Methods

Aerial parts of *Zornia brasiliensis* were collected in March 2016, the collection was carried out in Serra Branca, state of Paraíba. The botanical identification was carried out by Prof. Dr. José Iranildo Miranda de Melo from the State University of Paraíba (UEPB). An exsiccata was deposited in the Herbarium Arruda Câmara (ACAM) of Campus I UEPB, under code 1862.

The botanical material (5 kg) was dehydrated using a circulating air oven at 45 °C for 72 hours. After this, the material (3 kg) was shaken in a mechanical mill to obtain the powder. Then the powder was subjected to a thorough maceration with 95% ethanol (EtOH) for 72 hours in a stainless steel macerator, this process being repeated four times to obtain the extractive solution. The extractive solution was concentrated under reduced pressure on a rotary evaporator at a temperature of 40 °C to remove the solvent to give the crude ethanolic extract (CEE).

An aliquot of CEE (100.0 g) was subjected to vacuum liquid chromatography (VLC) with 300 g

of silica (ART 7734, MERCK, 0.060 – 0.200 mm e 70 - 230 mesh ASTM) deactivated with hexane, dichloromethane, ethyl acetate and methanol solvents. After fractionation the extractive solutions resulting from this process were concentrated in a rotary evaporator to give 0.73 g of the hexane fraction; 20.0 g of the dichloromethane fraction; 15.97g of the acetate fraction; 5.96 g of the 10% acetate-methanol fraction (AcOEt-MeOH 10%); and 59.81 g of the 50% acetate-methanol fraction (AcOEt-MeOH 50%).

An aliquot of 20 g of the 50% acetate-methanol fraction was dissolved in 300 ml of distilled water. It was then partitioned with n-butanol (300 mL) three times. The extractive solution of the n-butanolic phase was then concentrated on rotary evaporator under reduced pressure at a temperature of 50° C. After obtaining the dried material, it was dissolved in a solution with methanol-ethyl acetate (1:5, v/v) and then methanol was added until the precipitation of the saponins occurred. After precipitation the material

was allowed to decant for 72 hours at room temperature. The precipitate was then resuspended in methanol (200 mL) and concentrated on a rotary evaporator under reduced pressure at 40° C. Thus, obtaining a fraction rich in saponins and being encoded with FrST (1.1 g) [5].

A Shimadzu (Prominence) CLAE equipped with LC-20AT binary solvent pumping module, SIL-20A autoinjector, a degassing system DGU-20A, SPD-M20A diode array detector and CBM-20A were used. The column used was Phenomenex Gemini® C18 (250 mm x 4.6 mm i.d., 5 µm particles), with SecurityGuard Gemini® C18 pre-column (4 mm x 3.0 mm i.d., 5 µm particles). Method used for HPLC-DAD-MS / MS was water (0.1% formic acid) (A) and methanol (B). Gradient: 70% B (0.01 min) to 80% B (50.0 min), returning to 70% B (65.0 min) and remaining with 70% B to 80.0 min. Flow of the

mobile phase: 0.6 mL / min. Injection volume: 5 µL. Detection: 205 nm.

The low resolution mass spectrometer of Bruker, model Ion Trap-amaZonX using the technique of ionization by Eletrospray. The parameters of the Ion-Trap analysis were: capillary 4.5 kV, ESI in negative mode, end plate offset 500 V, nebulizer 24.5 psi, dry gas (N<sub>2</sub>) with flow of 4.5 L/h and temperature of 200° C. CID fragmentation was achieved in auto MS/MS mode using enhanced resolution mode for MS and MS/MS mode. The spectra (*m/z* 50-1000) were recorded every 2 sec. The high-resolution mass spectrometer model micrOTOF II (Bruker) using the Eletrospray Ionization technique. The micrOTOF II analysis parameters were: capillary 4.0 kV, ESI in the negative mode, end plate offset 500 V, 29.5 psi nebulizer, dry gas (N<sub>2</sub>) with flow of 7.0 L/h and temperature of 200° C. The spectra (*m/z* 50-1000) were recorded every 2 sec.

#### 4. Conclusions

This work enabled the development of an analytical methodology by HPLC-DAD-MS/MS that enabled the suggestion of the presence of Soyasaponin IV in *Zornia brasiliensis*. This being the first report of saponins in this genus. In this

way contributing with the chemistry *Z. brasiliensis* and corroborating with the quimitaxonomia of the family Leguminosae.

#### Acknowledgments

This study was supported by the Graduate Degree Program in Natural Products and Bioactive Synthetic Compounds and sponsored by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

#### Author Contributions

YN, LA, RL, participated in phytochemical analysis. MS and JT participated in interpretation of the mass spectra. JIMM participated in botanical identification.

#### Conflicts of Interest

The authors declare no conflict of interest.

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